

B²
C¹
B³

X PreCission protease cleavage buffer (50 mM TrisHCl, pH 7.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT) to remove unbound proteins, the fusion protein on the resin is cleaved with 100 units of HRV-3C protease (PreCission protease, 2 units/ μ l, from Pharmacia) by rocking on an inversion shaker for 16 hours at 4°C. The resin was spun down at 2,000 rpm for 10 min in a Sorvall RC-32 centrifuge. The supernatant and three washings (10 ml 1 X PBS per 10 ml resin), which contain the recombinant MUC18 protein, are then combined and concentrated by centrifuging through a Centricon-30 (Millipore/Amicon). The purity of the protein is characterized by SDS-PAGE (8 to 10% polyacrylamide gel, slab gel). The 70kDa contaminated protein is removed by passing through a Superdex 200 HR 10/30 column in 1 X PBS (void volume about 7 ml for a 20 ml packed column), and the fractions containing the recombinant middle fragment MUC18 protein (22 kDa) (eluted at about 15.5 ml) were pooled. Figs. 2A and 2B show the SDS-PAGE results of recombinant huMUC18 protein in the GST-fusion system.

In the Claims:

- C¹
B³
2. (Once amended) The method of claim 20, wherein said prostate cancer cell is from a biopsy tissue sample from a patient for whom a prediction of metastasis of prostate cancer is sought.

Please cancel claim 3 without prejudice.

- B⁴
4. (Twice amended) The method of claim 20, wherein expression of the MUC18 coding sequence is determined by immunoassay using antibody that recognizes and binds specifically to an epitope of MUC18 wherein said antibody is made in an experimental animal in response to the MUC18 antigen consisting of the amino acid sequence set forth in SEQ ID NO:2.

- SUB
C² → 15.
- (Twice amended) The method of claim 4, wherein the MUC18 antigen is a middle portion of the MUC18 polypeptide and consists of the amino acid residues of 211-376 of the amino acid sequence as set forth in SEQ ID NO:2.

Please cancel claims 13-19 without prejudice.

20. (Twice amended) A method for predicting an increased risk for metastasis of a prostate cancer cell that expresses a MUC18 coding sequence, said method comprising the steps of:

- B^S
- a) measuring the levels of expression of the MUC18 coding sequence in both the prostate cancer cell and a normal prostate cell,
 - b) comparing the levels of expression of the MUC18 coding sequence in the prostate cancer and normal cells, wherein higher level of expression of the MUC18 coding sequence in the prostate cancer cell relative to the level of expression in the normal prostate cell is positively correlated with an increased risk for metastasis.